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Susan J. Chivers, Robin W. Baird,
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David Matilla, Daniel J. McSweeney,
Erin M. Oleson, Carol L. Palmer,
Victoria Pease, Kelly M. Robertson,
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14. ABSTRACT

Chivers et al. (2007) found Hawai?i insular false killer whales to be distinct from other strata within the Indo-Pacific Ocean using mitochondrial DNA (mtDNA) control region sequence data. Here, we add new samples and eight nuclear DNA (nDNA) microsatellite markers to that study. After extensive quality checking, some haplotypes and duplicate individuals were removed from the 2007 mtDNA data set. A strong phylogeographic signal consistent with local haplotype evolution was evident for Hawai?i insular false killer whales with all but one individual having one of 2 closely related haplotypes found only in this population. The mtDNA characteristics of the Hawai?i insular false killer whales (n = 81) differed significantly (all p-values for Fisher exact and ΦST <0.0001) from both broad-scale strata (Central North Pacific (n = 13) and Eastern North Pacific (n = 39)), and all fine-scale strata (Hawai?i pelagic (n = 9), Mexico (n = 19), Panama (n = 15) and American Samoa (n = 6)). The magnitude of mtDNA differentiation (all ΦST >0.68) was consistent with less than one migrant per generation. The nDNA marker results were highly significant with all Fisher exact p-values &#8804 0.001 for comparisons of the Hawai?i insular stratum (n = 69) to the broad-scale strata (Central North Pacific (n = 13) and Eastern North Pacific (n = 36)), and fine-scale strata (Hawai?i pelagic (n = 9), Mexico (n = 19), Panama (n = 12) and American Samoa (n = 6)). The magnitude of differentiation was much less for nDNA (0.01 < FST < 0.08, 0.01 < Jost?s D < 0.06) than for mtDNA, indicating the potential for some male-mediated gene flow although the possibility that FST is low because of the high mutation rate of microsatellites or the influence of selection operating to counter gene flow cannot be excluded. Inferences from these data are limited by sample distribution, with pelagic false killer whales near the Hawaiian Islands inadequately sampled. However, the small number of Hawai?i insular false killer whales (around 120) together with an estimated effective population size of 44.3 (95% CI = 31.2-67.2) are causes for concern about loss of genetic diversity.

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EVIDENCE OF GENETIC DIFFERENTIATION FOR HAWAI`I INSULAR FALSE KILLER WHALES *(Pseudorca crassidens)*

Susan J. Chivers[1], Robin W. Baird[2], Karen M. Martien[1], Barbara L. Taylor[1], Eric Archer[1], Antoinette M. Gorgone[3], Brittany L. Hancock[1], Nicole M. Hedrick, David Matilla[4], Daniel J. McSweeney[5], Erin M. Oleson[6], Carol L. Palmer[7], Victoria Pease[1], Kelly M. Robertson[1], Jooke Robbins[8], Juan Carlos Salinas[1,9], Gregory S. Schorr[2], Mark Schultz[10], Janet L. Thieleking[1], and Daniel L. Webster[2]

[1]NOAA Southwest Fisheries Science Center, 3333 N. Torrey Pines Court, La Jolla, CA 92037 USA
[2]Cascadia Research Collective 218 ½ W. 4th Avenue, Olympia, WA 98501 USA
[3]NOAA Southeast Fisheries Science Center, 101 Pivers Island Road, Beaufort, NC 28516 USA
[4]Hawaiian Islands Humpback Whale National Marine Sanctuary, 726 S. Kihei Road, Kihei, HI 96753 USA
[5]Wild Whale Research Foundation, Box 139, Holualoa, HI 96725 USA
[6]NOAA Pacific Islands Fisheries Science Center, 2470 Dole St., Honolulu, HI 96822 USA
[7]Department of Natural Resources, Environment, The Arts and Sport, Palmerston, NT 0831 Australia
[8]Provincetown Center for Coastal Studies, 5 Holway Avenue, Provincetown, MA 02657 USA
[9]Departamento de Biologia Marine, UABCS, AP 19-B La Paz, BSC Mexico CP 23081
[10]Charles Darwin University, Arafura Timor Research Facility, Casuarina NT 0811, Australia

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Abstract

Chivers et al. (2007) found Hawai‘i insular false killer whales to be distinct from other strata within the Indo-Pacific Ocean using mitochondrial DNA (mtDNA) control region sequence data. Here, we add new samples and eight nuclear DNA (nDNA) microsatellite markers to that study. After extensive quality checking, some haplotypes and duplicate individuals were removed from the 2007 mtDNA data set. A strong phylogeographic signal consistent with local haplotype evolution was evident for Hawai‘i insular false killer whales with all but one individual having one of 2 closely related haplotypes found only in this population. The mtDNA characteristics of the Hawai‘i insular false killer whales (n = 81) differed significantly (all p-values for Fisher exact and Φ_{ST} <0.0001) from both broad-scale strata (Central North Pacific (n = 13) and Eastern North Pacific (n = 39)), and all fine-scale strata (Hawai‘i pelagic (n = 9), Mexico (n = 19), Panama (n = 15) and American Samoa (n = 6)). The magnitude of mtDNA differentiation (all Φ_{ST} >0.68) was consistent with less than one migrant per generation. The nDNA marker results were highly significant with all Fisher exact p-values ≤ 0.001 for comparisons of the Hawai‘i insular stratum (n = 69) to the broad-scale strata (Central North Pacific (n = 13) and Eastern North Pacific (n = 36)), and fine-scale strata (Hawai‘i pelagic (n = 9), Mexico (n = 19), Panama (n = 12) and American Samoa (n = 6)). The magnitude of differentiation was much less for nDNA (0.01 < F_{ST} < 0.08, 0.01 < Jost’s D < 0.06) than for mtDNA, indicating the potential for some male-mediated gene flow although the possibility that F_{ST} is low because of the high mutation rate of microsatellites or the influence of selection operating to counter gene flow cannot be excluded. Inferences from these data are limited by sample distribution, with pelagic false killer whales near the Hawaiian Islands inadequately sampled. However, the small number of Hawai‘i insular false killer whales (around 120) together with an estimated effective population size of 44.3 (95% CI = 31.2-67.2) are causes for concern about loss of genetic diversity.

Introduction

Two stocks of false killer whales (*Pseudorca crassidens*) are recognized within the Hawaiian Exclusive Economic Zone (EEZ): the Hawai‘i insular and Hawai‘i pelagic stocks (Carretta et al. 2010). The Hawai‘i insular stock of false killer whales is estimated to be 123 (CV = 0.72) individuals (Baird et al. 2005). Two lines of evidence were used to support recognition of the Hawai‘i insular stock as a demographically independent population: 1) genetic results using mitochondrial DNA (mtDNA), which indicated restricted gene flow between false killer whales sampled around the main Hawaiian Islands and false killer whales sampled in other discrete locations in the eastern North Pacific Ocean (Chivers et al. 2007), and 2) photo-identification and satellite tagging results, which revealed long-term site fidelity of false killer whales within the near shore waters of the main Hawaiian Islands (Baird et al. 2008, 2010).

Genetic data can be used to reveal different levels of units to conserve (Taylor 2005). Chivers et al. (2007) focused on delineating Demographically Independent Populations (DIPs), which are relevant to the ecological time-scale pertinent to the U.S. Marine Mammal Protection Act (MMPA). The goal of the MMPA is to maintain population stocks as functioning elements of their ecosystem. Genetic data can also be used to reveal structure at longer time scales such as the evolutionary timescales pertinent to defining species, subspecies and Distinct Population Segments (DPSs) (NMFS 1996). In this case, the magnitude of genetic differentiation between DPSs is expected to be large (i.e., consistent with less than one migrant per generation) and phylogeographic patterns (i.e., haplotypes or genotypes that are found nearly exclusively in one region indicating significant reproductive isolation) may be present.

This report incorporates data from additional samples collected from the Hawai‘i insular stock and several locales within the Pacific to update the mtDNA control region genetic study of Chivers et al. (2007). We also add eight microsatellite loci, nuclear DNA (nDNA) markers, to identify duplicate samples and to estimate differentiation from other locales. Our results support the previous conclusion that the Hawai‘i insular stock is demographically independent and provides information about the evolutionary significance of this stock. Because we are investigating the genetic differentiation of Hawai‘i insular false killer whales relative to other false killer whales beyond the specific application of the MMPA, we will refer to this group as Hawai‘i insular false killer whales and not further use the term “stock”.

We also estimate the effective population size (N_e) of Hawai‘i insular false killer whales. Wright (1931, 1938) defined N_e as the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration. Effective population size is directly related to genetic diversity and hence the amount of genetic variability that a population has available to respond to various environmental challenges. Populations that drop to low abundance and stay at low abundance will inevitably lose genetic diversity. Effective population size is also affected by fluctuations in abundance and social structure. For example, a mating system where some males father many offspring while others father few to none would have a lower effective population size than a random mating system where all males have an equal chance of passing on their genes. Hawai‘i insular false killer whales have both a low estimated current abundance and strong social structure (Baird et al. 2008), though details of the mating system remain unknown. Because loss of genetic diversity contributes to the risks facing small populations (Lande 1988), we calculate N_e and discuss the implications.

Materials and Methods

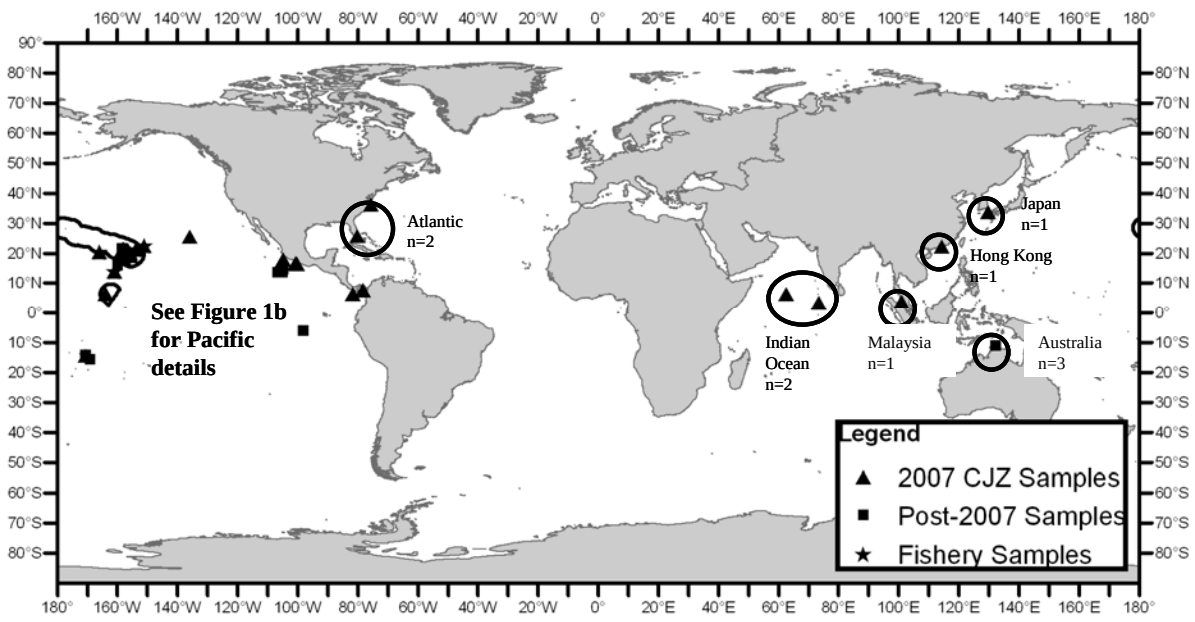
The Samples

The sample set consisted of 189 tissue samples collected from false killer whales biopsied at-sea (n = 185 including 7 sampled by observers during long-line fishing operations) or stranded on the beach (n = 4) between 1983 and 2009 (Figs. 1 and 2). Most of these samples (n = 154) were collected from 28 groups of false killer whales (Table 1). For each group encountered, estimates of group size were recorded and photographs for individual identification purposes were taken of as many individuals as possible. Samples were considered part of the Hawai‘i insular stratum if they were collected from a group containing any individuals that were part of the Hawai‘i insular social network as determined by analyses of the photo-identification catalog and association pattern data (Baird et al. 2008; Baird 2009). All tissue samples (i.e., skin or muscle) were preserved frozen or in a 20% dimethylsulphoxide solution saturated with NaCl (Amos and Hoelzel 1991; Amos 1997) and archived in the Southwest Fisheries Science Center's (SWFSC) Protected Species Tissue Collection.

DNA extraction, PCR Amplification and Sequencing

The 5' end of the hypervariable mtDNA control region was amplified from extracted genomic DNA (lithium chloride protocol: Gemmell and Akiyama 1996; sodium chloride protocol: Miller et al. 1988; Qiagen DNeasy protocol #69506) using the polymerase chain reaction (PCR) and then sequenced using standard techniques (Saiki et al. 1988; Palumbi et al. 1991). DNA was amplified using a 25ul reaction of 1ul DNA, 18ul of water, 2.5 ul of buffer [10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 µl of 10 mM dNTP], 0.75 µl of each 10 µM primer, and 0.5 units of Taq DNA polymerase. The PCR cycling profile consisted of 90 °C for 2.5 min, followed by 35 cycles of 94 °C for 50 sec, an annealing temperature of 60 °C for 50 sec, and 72 °C for 1.5 min, then a final extension of 72 °C for 5 min. The sequence was generated in two parts. For the first segment, we used primers H16498 (5'-cctgaagtaagaaccagatg- 3') (Rosel et al. 1994) and L15829 (5'-cctccctaagactcaagg- 3') (developed at the SWFSC), and for the second segment, we used primers H497 (5'-aaggctaggaccaaacct- 3') and L16218 (5'-tggccgctccattagatcacgagc- 3') (both developed at the SWFSC). The light and heavy strands of the amplified DNA product for each specimen were sequenced independently as mutual controls using standard four color fluorescent protocols on the Applied Biosystems Inc. (ABI, Foster City, CA) model 377, 3100 and 3730 sequencers. The second segment of approximately 573 base pairs included an approximately 20-base-pair section of overlap with the first 395 base pairs of the control region to ensure all sequences were complete. The final sequences were 947 base pairs long and were aligned using SEQED, version 1.0.3 (ABI) and Sequencher software (versions 4.1 and 4.8; Gene Codes, Ann Arbor, MI).

(a)



(b)

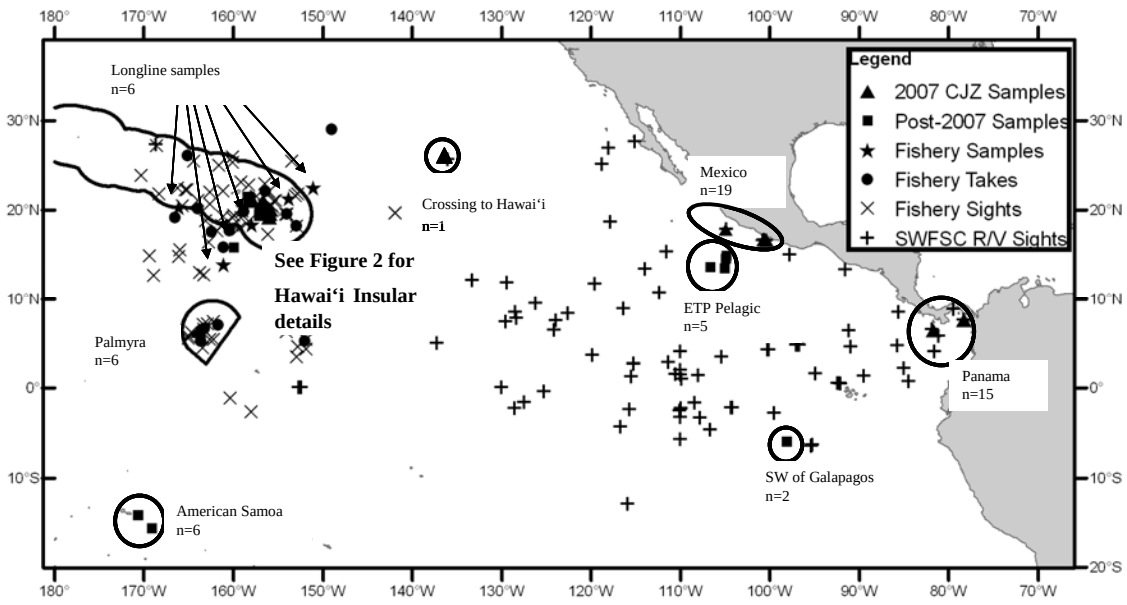
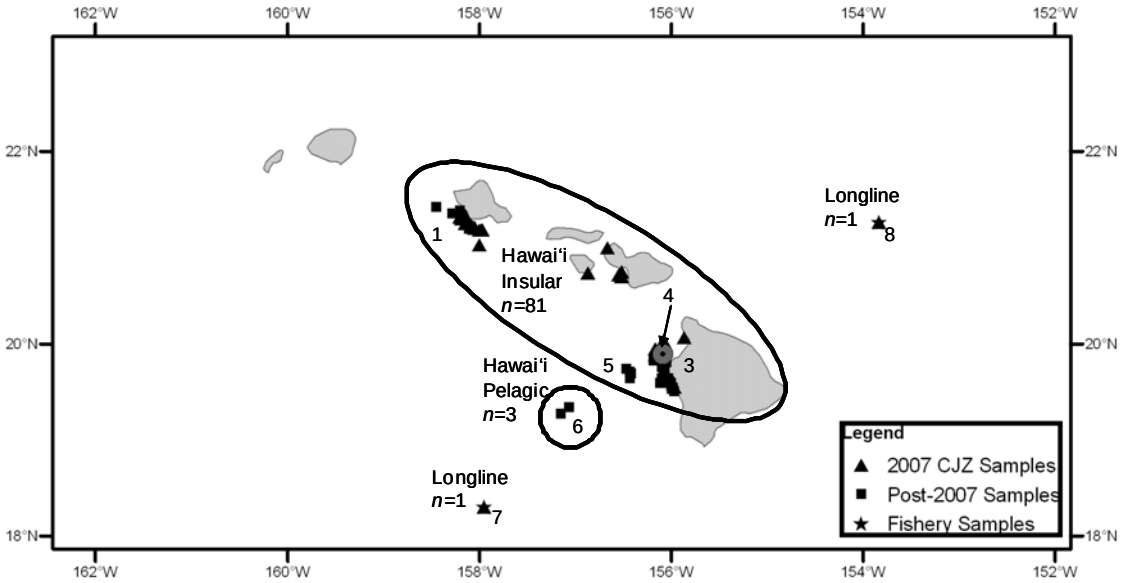


*Figure 1. (a) Collection locations of all samples used in our study. (b) Samples collected in the eastern Pacific Ocean are shown here with research and fishing vessel sightings of false killer whales (*Pseudorca crassidens*). The sightings data were collected on aerial and shipboard surveys conducted between 1974 and 2005 (Mobley et al. 2000; Baird et al. 2005; Gerrodette and Forcada 2005; Barlow 2006) and by observers working aboard long-line fishing vessels between 1994 and 2004 (NOAA, NMFS, PIRO). See legend for guide to symbols; the fishery collected samples indicated by a solid star. All other collection locations for samples used in Chivers et al. (2007) are indicated by a solid triangle, with the non-fishery samples added to this analysis indicated by a solid square. The exclusive economic zones around the Hawaiian islands and the Palmyra Atoll are shown for reference.*



Map #	Haplotypes represented	Number of individuals/haplotype	Number of groups sampled
1	1, 2	24, 2	6
2	1, 2	9, 3	4
3	1, 2	20, 8	9
4	1, 2, 5	5, 4, 1	1
5	1	5	1
6	9, 25	1, 2	1
7	9	1	1
8	6	1	1

Figure 2. Detailed information about the skin-biopsy samples collected from false killer whales around the main Hawaiian Islands. See legend and corresponding table for detailed information about the samples collected. Haplotype numbers correspond to the haplotype numbers used in Fig. 4.

Table 1. Characteristics of the false killer whale groups with multiple samples collected. The 'mean' group size is the mean of multiple best estimates of group size recorded for each group; the range given in parentheses is the minimum and maximum of all estimates of group size. The '# of individuals' is the number of distinct individual animals sampled from the group after data were reviewed to remove duplicate samples. These individuals (together with the singleton samples from other groups sampled and not listed here) were included in analyses of the mitochondrial DNA sequence data. The name of the smallest-scale stratum to which each group belongs is identified under each regional heading.

	Group	Group size: mean (range)	# of samples collected	# of individuals	# of haplotypes	Sex (Females, Males, Unknown)
Hawai'i Insular						
Maui	1	18 (15-24)	4	2	2	0, 2, 0
Maui	2	14 (11-17)	3	2	1	1, 1, 0
Maui	3	35 (30-50)	6	6	2	3, 3, 0
O'ahu	4	5 (5-5)	4	3	1	3, 0, 0
Hawai'i	5	41 (38-50)	7	7	2	4, 3, 0
O'ahu	6	35 (30-40)	22	18	1	8,10, 0
Hawai'i	7	41 (38-50)	2	1	1	1, 0, 0
Hawai'i	8	30 (12-70)	10	10	3	5, 3, 2
Hawai'i	9	9 (8-12)	2	2	1	1, 1, 0
Hawai'i	10	30 (20-40)	7	5	2	3, 2, 0
Hawai'i	11	20 (16-26)	4	4	2	4, 0, 0
Hawai'i	12	13 (13-15)	5	5	1	3, 2, 0
Hawai'i	13	20 (20-24)	4	1	1	0, 1, 0
Hawai'i	14	14 (11-16)	7	7	1	4, 3, 0
O'ahu	15	15 (12-19)	2	2	1	2, 0, 0
O'ahu	16	20 (17-25)	2	1	1	0, 1, 0
Eastern Pacific						
Mexico	1	20 (18-29)	5	4	1	3, 1, 0
Panama	2	26 (21-32)	13	12	1	5, 7, 0
Panama	3	17 (15-20)	3	3	1	1, 2, 0
Mexico	4	51 (32-64)	8	6	2	2, 4, 0
Mexico	5	8 (5-14)	7	6	1	5, 1, 0
Mexico	6	10 (7-13)	7	3	1	2, 1, 0
Offshore	7	32 (23-57)	3	2	1	0, 2, 0
Offshore	8	17 (12-28)	2	2	1	0, 2, 0
Offshore Hawai'i	9	18 (10-30)	2	2	1	2, 0, 0
Palmyra Atoll	10	17 (11-50)	6	3	2	3, 0, 0
American Samoa	11	N/A (10 – 20)	4	4	1	2, 2, 0
Australia	1	30 (N/A)	3	3	1	0, 0, 3

Nuclear DNA processing

Samples were genotyped using microsatellite DNA primers for eight dinucleotide loci: D12t derived from beluga whales (*Delphinapterus leucas)* (Buchanan *et al.*, 1996), EV94t derived from humpback whales (*Megaptera novaenglia*) (Valsecchi and Amos 1996), KWM2at, KWM2b, KWM12at derived from killer whales (*Orcinus orca*) (Hoelzel et al., 1998a), and Ttr11, Ttr34, and Ttr48 (Rosel et al. 2005) derived from common bottlenose dolphins (*Tursiops truncatus*). Extracted DNA was amplified using the PCR in 25 µl reactions containing 1 µl (approximately 5-50 ng) genomic DNA, 18.25 µl water, 2.5 µl of buffer [10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$], 0.75 µl of each 10µM primer, 1.5 µl 10mM dNTP and 0.5 units of *Taq* DNA polymerase. The PCR thermal cycling profile for these primers was 90 °C for 2.5 min, followed by 35 cycles of 94 °C for 45 sec, 1 min at annealing temperature, and 72 °C for 1.5 min, then a final extension of 72 °C for 5 min. The optimal annealing temperatures were 48 °C for KWM2at and KWM2b, 50 °C for KWM12at, 55 °C for D12t, EV94t and Ttr11, and 57 °C for Ttr34 and Ttr48.

Size and purity of the amplicon were assessed electrophoretically. Genotype data were generated on ABI genetic analyzers (models 3100 and 3730) using a commercial internal lane standard (ROX500®; PE Applied Biosystems Inc.). ABI's GENEMAPPER (version 4.0) software was used to make preliminary allele fragment size 'calls.' GENEMAPPER's calls were reviewed and, if necessary, edited by a trained genotyper before the calls were finalized. Data generated on the ABI 3100 were normalized from runs of a set of samples on the ABI 3730 using the program Allelogram (Morin et al. 2009b). The size of each allelic pair for each locus constituted the raw data for analyses.

Sex determination

Samples were genetically sexed using the zinc finger (ZFX and ZFY) genes. Prior to 2005, sex determinations were completed according to Fain and LeMay (1995). After 2005, a Real-Time PCR (Stratagene) assay was used as described in Morin et al. (2005).

Data review

A minimum of 10% of the sample set was replicated for each marker during data generation, and these records were reviewed for consistency in allele size scoring. After data generation had been completed, all allele size calls were reviewed by an independent trained genotyper. The independent genotyper used GENEMAPPER to re-analyze all of the genetic analyzer raw datafiles and call all allele sizes, just as the original genotyper did. The independent genotyper's calls were then normalized by people not involved with generating or normalizing the original data set. This resulted in a second complete data set that, while based on the same raw data files as the original data set, represented completely independent calls of the raw data. Inconsistencies between the original data set and that generated by the independent

genotyper were jointly reviewed by both genotypers and, where possible, resolved. If the genotype of a sample could not be fully resolved at a given marker, that genotype was left blank and treated as missing data.

Prior to analyses, the final nDNA data set was reviewed for quality (Morin et al. 2009c; see Appendix 2 for a more detailed description of the data quality assurance steps and results). Samples that could not be consistently replicated, were missing data for >25% of the markers or were homozygous at six or more loci were deemed to be of poor quality and removed from the data set. The program Microchecker (version 2.2.3; Van Oosterhout et al. 2004) was used to examine the markers for allelic dropout and null alleles. Deviation from Hardy-Weinberg equilibrium (HWE) was assessed for each microsatellite locus using GenePop version 4 (Rousset 2008). Both exact tests of HWE (Guo and Thompson 1992) and tests for heterozygote deficiency (Raymond and Rousset 1995) were conducted. The same software was used to evaluate linkage disequilibrium for each pair of loci using Fisher's method and the Markov chain method. All HWE and linkage disequilibrium tests were conducted using program defaults for the Markov chain parameters (1,000 dememorization steps, 100 batches, 1,000 iterations per batch). The tests were conducted separately for samples from the Hawai'i insular, Mexico, and Panama strata (see below for definitions), as these were the only strata with sufficient samples. Fisher's method (Fisher 1935) was used to combine *p*-values across strata to calculate a global *p*-value for each locus.

The jackknife procedure described in Morin et al. (2009a) was used to identify samples that were highly influential in deviations from HWE. Genotypes that had log-odds larger than two were removed from the data set.

Pairs of samples that matched in sex, mtDNA haplotype, and microsatellite genotype were considered duplicate samples, and only one sample was kept in the final data set. When available, photo-identification data were also used to identify duplicate samples from the same individual. The programs Dropout (McKelvey and Schwartz 2005) and MSTools (Park 2001) were used to identify additional pairs of samples whose genotypes differed at four or fewer loci. These pairs could represent duplicate samples with genotyping errors. We used the program GenAlEx v.6 (Peakall and Smouse 2006) to examine our ability to discriminate unique individuals using our microsatellite data set. We calculated both the probability that two randomly chosen individuals would possess the same multi-locus genotype (PI) and the probability that full-siblings would share the same genotype (PIsibs; Taberlet and Luikart 1999).

We reviewed the haplotype data published by Chivers et al. (2007) to ensure data quality. All samples with unique haplotypes (i.e., not present in any other sample) were re-sequenced two or more times to confirm the sequence.

Error rate estimation

The nDNA data used in this study was generated at two different times. The first 113 samples were genotyped in 2006 and 2007, while the remaining samples were genotyped in 2010. For the 2006 and 2007 data, more than ten percent of the genotypes were replicated. However, that replication was a combination of random replication and systematic replication

(e.g., poor-performing samples that were run multiple times to verify data and well-performing samples that were used as controls across experiments). No records are available to determine which samples were included in the random replication. For the 2010 data, on the other hand, ten percent of the samples were chosen for random replication at the start of the process. We used those samples to calculate a genotyping error rate for the 2010 data. Each allele call among the replicated samples was compared to the call used in the final data set. All discrepancies were considered errors. The number of errors was divided by the total number of allele calls for the replicated samples to produce a per-allele genotyping error rate for the 2010 data.

To calculate an overall error rate for the entire nDNA data set, we compared allele size calls generated by the independent genotyper to the final data set used in our analyses. We did not use the data generated by the original genotypers for this comparison because that data set was generated over the course of several years, during which many of the errors inherent in data generation (e.g., sample mix-ups, mis-called alleles, errors introduced during data editing and manipulation) had been discovered and corrected. Estimating the error rate after these corrections were made would under-estimate the error rate. The independent genotyper's data set, on the other hand, was generated over the course of a few weeks, and no changes or corrections were made to it after the allele sizes were called and normalized. All allele size calls made by the independent genotyper, including all replicate calls for genotypes that were replicated in the lab, were compared to the allele size that was used in the final data set. All discrepancies, including alleles that were called by the independent genotyper but set to null in the final data set or vice versa, were considered errors. The number of errors was divided by the total number of allele calls made by the independent genotyper, including all calls of replicates, to produce a final per-allele genotyping error rate.

Analyses of genetic data

Genetic diversity – We identified haplotypes and quantified genetic variability in terms of haplotypic diversity (*h*) and nucleotide diversity (π) using Arlequin, version 3.11 (Excoffier et al. 2005). We also used Arlequin to calculate Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) for the Hawai‘i insular stratum to look for evidence of population expansion or a bottleneck. For the nDNA data set, the program FSTAT (Goudet 2001) was used to calculate allelic richness and number of alleles per locus, while GenePop (Raymond and Rousset 1995) was used to calculate observed heterozygosity.

Phylogeographic structure – A minimum spanning network was generated using the program Arlequin, version 3.11 (Excoffier et al. 2005) to examine the concordance between the distribution of mtDNA control region haplotypes and geographic region for evidence of the role evolutionary processes may have played in the patterns revealed. Optimal minimum spanning networks incorporate information about haplotype frequency to obtain the most parsimonious network for haplotype evolution. For example, haplotypes that are 'rare' or occur at low frequencies would be most likely to have been derived from haplotypes that are 'common' or occur in high frequencies rather than from another 'rare' haplotype for a given series of mutation events (Excoffier and Smouse 1994; Excoffier et al. 1992).

Assignment test – We used the program GeneClass2 (Piry et al. 2004) to investigate the origin of the animals sampled from one group about 12 km off the island of Hawai‘i (group 4 in Fig. 2), as one sample from this group had a haplotype not closely related to the other Hawai‘i

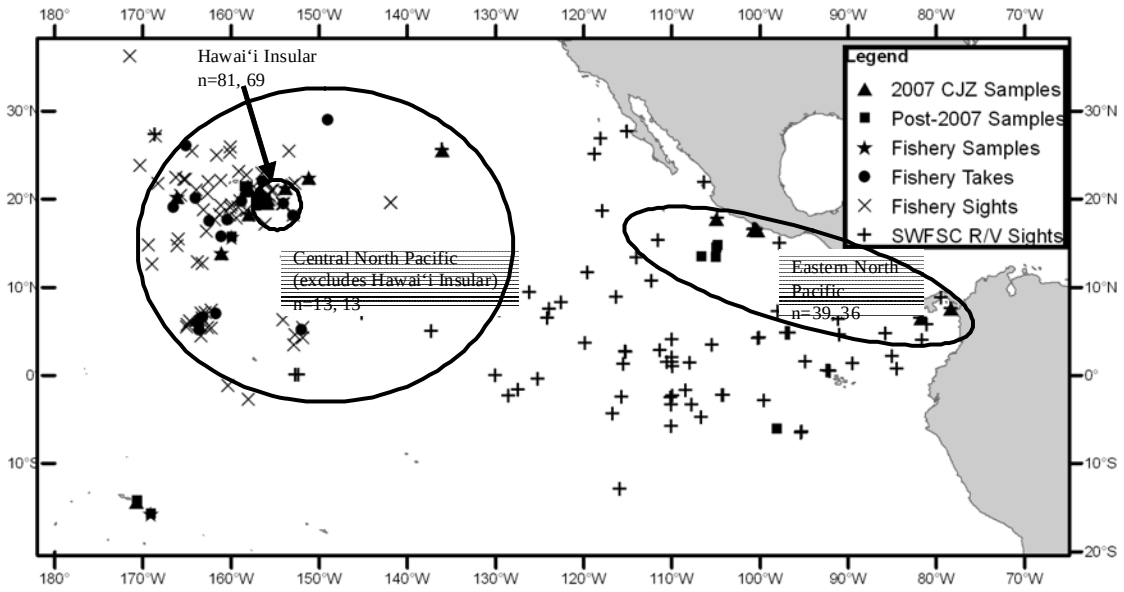
insular haplotypes (see results). We used the nDNA data set and the assignment criterion of Paetkau et al. (1995) to calculate the likelihood of each individual sampled from that group having originated in the Hawai‘i insular stratum and assessed the significance of those likelihoods using Paetkau et al.'s (2004) re-sampling method. The reference data used for the assignment test was all Hawai‘i insular samples except those from group 4. To assess the power of this analysis to exclude the Hawai‘i insular false killer whales as the possible origin of non-resident individuals, we repeated this analysis using all Hawai‘i insular samples (including group 4) as the reference data set and calculated assignment probabilities for all samples from the American Samoa and Hawai‘i pelagic strata (see below for strata definitions) and one group each from Mexico and Panama. For all analyses, we set the default frequency for missing alleles at 0.01, performed 1,000 re-sampling events, and set the type I error rate to 0.01 as recommended by Piry et al. (2004).

Genetic differentiation – Conventional analyses for detecting population structure by quantifying genetic differentiation among putative populations were also conducted. We tested the null hypothesis of panmixia for three *a priori* data stratifications of the data set. In the first *a priori* stratification, called the broad-scale stratification, we recognized three strata: Hawai‘i insular, Central North Pacific (CNP), and Eastern North Pacific (ENP) (Fig. 3a). In the second *a priori* stratification, called the fine-scale stratification, we recognized five strata: Hawai‘i insular, Hawai‘i pelagic, Mexico, Panama, and American Samoa (Fig. 3b). This stratification differs from the Chivers et al. (2007) analyses in that the Palmyra Atoll stratum is excluded due to small sample size (n = 3), and the American Samoa stratum is added. The third *a priori* stratification, referred to as the inter-island stratification, recognized each of the three main Hawaiian Islands (O‘ahu, Maui and Hawai‘i) where samples were collected, as in Chivers et al. (2007) (Fig. 2).

For each stratification, we conducted both global and pairwise tests of the null hypothesis of no population structure among strata by conducting a global Fisher's exact test of differentiation (Raymond and Rousset 1995), as implemented in Arlequin (Excoffier et al. 2005), for the mtDNA sequence data set and using a χ^2 test (Rolf and Bentzen 1989) for the nDNA data set. The χ^2 test was implemented using custom code (available upon request) written in the statistical programming language R (R Core Development Team, 2006). Fisher's exact test has been shown to be more powerful than an F_{ST} or Φ_{ST} permutation test for evaluating statistical significance in mtDNA data sets (Hudson et al. 1992), while the χ^2 test is more powerful than F_{ST} permutation tests for microsatellites (Goudet et al. 1996). Statistical significance was determined from 10,000 random permutations of each data set.

Pairwise estimates of genetic divergence between strata were calculated using Φ_{ST} for the mtDNA sequence data, and both F_{ST} (Wright 1931; Weir and Cockerham 1984) and Jost's D (Jost 2008) for the nDNA microsatellite data. D is a measure of genetic divergence that, unlike F_{ST}, is independent of genetic diversity and is therefore expected to produce more accurate estimates of divergence for highly polymorphic markers like microsatellites (Jost 2008). Φ_{ST} was calculated using the program Arlequin (Excoffier et al. 2005), with the number of homologous nucleotide differences between two individuals as the measure of genetic distance. F_{ST} and Jost's D were calculated using custom code (available upon request) written in the statistical programming language R (R Core Development Team 2006). We evaluated the statistical significance of all divergence measures from 10,000 random permutations of each data sets.

(a)



(b)

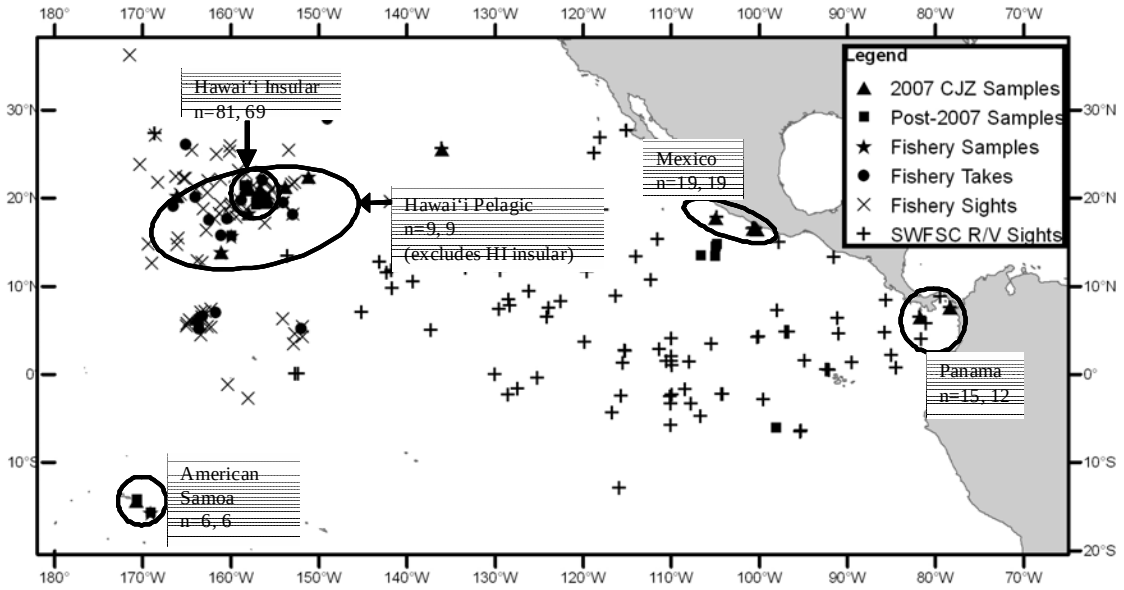


Figure 3. The false killer whale mitochondrial (mtDNA) and nuclear DNA (nDNA) data sets were analyzed as shown (a) for the broad-scale and (b) fine-scale stratifications. Sample sizes for the mtDNA and nDNA data sets are presented, respectively.

The statistical significance of all differentiation and divergence results was interpreted with $\alpha = 0.05$. A multiple test correction factor was not applied to interpret results because (1) not all null hypotheses are expected to be true simultaneously and (2) the test effectively reduces the critical value (α), or Type I error rate, at the expense of the Type II error rate (Perneger 1998). The latter point, in particular, has conservation management implications for this study (see Dizon et al. 1995; Taylor et al. 1997).

To test for evidence of sex-biased dispersal by looking for living immigrants, we calculated F_{ST} between each pair of putative populations separately for males and females using the mtDNA and nDNA independently. We used a permutation test to determine whether differences in F_{ST} between sexes were statistically significant. Permuted data sets were generated for each stratum by randomly permuting sex among samples within that stratum. We calculated the absolute difference in F_{ST} between males and females in the permuted data set for each pairwise comparison. We repeated the permutation 1,000 times to generate a null distribution for the expected difference between F_{ST} for males and females for each pair of strata. The *p*-value for each pairwise comparison was calculated as the proportion of the null distribution that was greater than or equal to the observed difference in F_{ST} between the sexes. For this analysis, all F_{ST} values were calculated using the R package Hierfstat (Goudet 2006; R Development Core Team 2006), and the permutation was performed using custom R code (available upon request). This analysis was only used to test for sex-biased dispersal within Mexico, Panama, and the Hawai‘i insular strata and within the three main Hawaiian Islands (O‘ahu, Maui and Hawai‘i). Sample sizes were too small to consider other strata.

Effective population size – We estimated the effective population size (N_e) of the Hawai‘i insular false killer whales using the program LDNe (Waples 2006; Waples and Do 2010). LDNe uses estimates of linkage disequilibrium (LD) to infer the N_e. We set the lowest allele frequency to be used in the analysis at 0.05 and assumed random mating. Ninety-five percent confidence intervals were calculated using the jackknife procedure proposed by Waples (2006).

Bottlenecks – We used the analytical method described in Cornuet and Luikart (1996) and implemented in the program BOTTLENECK (Piry et al. 1999) to test for evidence of a recent decline in abundance within Hawai‘i insular false killer whales. The analysis takes advantage of the fact that when the effective size of a population is reduced, the allelic diversity of the population is reduced more rapidly than its heterozygosity, resulting in an apparent excess of heterozygosity given the number of alleles detected. We used BOTTLENECK to analyze nDNA from the Hawai‘i insular false killer whales under the two-phase model (TPM) of mutation with 95% single-step mutations, 5% multiple-step mutations, and variance among multiple steps of 12, as recommended by Piry et al. (1999). We only ran the sign test of statistical significance, as the standardized differences test performs poorly with fewer than 20 loci (Cornuet and Luikart 1996) and the methodology, performance, and correct interpretation of the Wilcoxon's test are undocumented (Piry et al. 1999). For comparative purposes, we also ran identical analyses of the nDNA for Mexico, which is the only other stratum from the fine-scale stratification with sufficient samples. All significance tests were based on 1,000 iterations.

Results

Data Review

The probability of identity was 3.18×10^{-9} for unrelated individuals and 7.05×10^{-4} for full-siblings, indicating that the microsatellite loci were adequate for identifying unique individuals. Using available photographs and genotypes from the eight nDNA markers, 24 duplicate samples from 22 individuals were identified and removed prior to analyses. Seven additional potential duplicate samples were identified, and results of analyses with these omitted are presented in Appendix 1.

In addition to the removal of duplicate samples, 14 samples were eliminated from the mtDNA analysis and 25 samples from the nDNA analysis due to poor sample quality. Ten samples included in the mtDNA data set were received too late to be genotyped and are not included in the nDNA data set. After these exclusions, the data sets used for all summary statistics and analyses included 151 samples in the mtDNA data set and 130 samples in the nDNA data set.

Two samples were identified in the HWE jackknife analysis as having likely genotyping errors. Both were homozygous for rare alleles, one at locus D12t and one at locus Ttr11. The genotypes of these samples at these loci were set to null for all analyses.

Locus D12t was out of HWE in the Hawai‘i insular stratum. No deviation was detected for any other loci in the Hawai‘i insular stratum, and no deviation was detected for loci in Mexico or Panama. There was no evidence of linkage disequilibrium in the data set. No loci showed evidence of null alleles or allelic dropout, according to the Microchecker analysis. Because deviations from HWE and linkage equilibrium are not unexpected in a small population from which more than half of the individuals have been sampled, like the Hawai‘i insular false killer whales, all markers were retained.

Re-sequencing of samples revealed errors in the Chivers et al. (2007) sequences and resulted in the elimination of seven haplotypes: 3, 4, 8, 13, 14, 15, and 22 (numbers correspond to the haplotype numbers in Fig. 4b). Thus, there were 17 rather than 24 haplotypes among the samples included in that study. The numbers assigned to those haplotypes were not re-used.

Genotyping error rate

For samples genotyped in 2010, 18 samples were randomly chosen for replication. These samples were genotyped an average of 2.3 times at each nuclear locus, for a total of 674 allele calls. Sixteen errors were detected, resulting in a per-allele error rate of 0.0237 for the 2010 samples. Comparison of the data set generated by the independent genotyper to the final data set revealed 147 discrepancies out of the 4,358 allele size calls made by the independent genotyper. This results in a per-allele error rate of 0.0337 for the entire data set.

Genetic diversity

There were 22 haplotypes identified among the 151 mtDNA sequences representing individual animals in the data set, including five additional haplotypes identified among the

samples added to the Chivers et al. (2007) data set. The mean number of pairwise differences between haplotypes was 3.45 (+/- 1.771), and there were 31 polymorphic sites including 29 substitutions (28 transitions and 1 transversion) and 2 insertions/deletions in the 947 base pair sequences (Table 2). The observed nucleotide diversity was low (i.e., $\pi = 0.36\%$; Table 3) compared to most other delphinids (i.e., 1-2%) but comparable to estimates of nucleotide diversity for sperm whales (*Physeter macrocephalus*, 0.38%, Lyrholm et al. 1996) and other closely related species (e.g., killer whales, *Orcinus orca*, 0.54%, Hoelzel et al. 1998a; short-finned pilot whales, *Globicephala macrorhynchus*, 0.84% in Oremus et al. 2009). Overall haplotypic diversity was 0.781 (+/- 0.028; Table 3).

Table 2. The polymorphic sites of 22 haplotypes identified for false killer whales are shown here. GenBank accession numbers are included next to each haplotype for those published in Chivers et al. (2007), and the Southwest Fisheries Science Center's Protected Species Tissue Collection Accession Number is provided for new haplotypes identified in this report. Sequencing errors found in haplotypes 3 (EF601199), 4 (EF601200), 8 (EF601203), 13 (EF601211), 14 (EF601212), 15 (EF601213), and 22 (EF601215) since Chivers et al. (2007) was published revealed that these individuals had haplotypes 1, 1, 9, 10, 9, 11 and 21, respectively. The haplotype numbers 3, 4, 8, 13, 14, 15, and 22 were not re-used, hence the series runs to number 29. Haplotype numbers correspond to those in Fig. 4.

		11222222 2223333333 334555677
		6907055567 8990000026 998045419
		6880702376 5072567890 451719291
Hawai'i Insular		
1	(EF601197)	TCTTCACCAC CTCGGCCCTC CCCCGCATG
2	(EF601198)	....T..T .....
5	(EF601201)	......T... ...A..T... ...T.T...
Eastern Pacific Ocean, except Hawai'i Insular population		
6	(EF601204)	......T..T ....A.T... .....
7	(EF601202)	......T... ...A..T... T....T...
9	(EF601207)	..... T..A..T... .....T...
10	(EF601208)	....TA..T... .....T...
11	(EF601209)	....T... .TTT.TG..
12	(EF601210)	......T... ...A.TT... T....T...
16	(EF601205)	.......T.. T..A..T... .....T...
17	(EF601206)	......T..T T.....T... ...T.T...
25	(73895)	...C..... ...A..TT.. T..T.T...
26	(74710)	.T....T... ..T...T... T..T.T...
27	(67067)	......T... ......T... .....T...
28	(67155)	....T.C. ...T.T...
29	(72696)	....TA..T... .....TG..
Central Indian Ocean		
21	(EF601214)	....A..T... T..T.T...
Western Pacific Ocean		
18	(EF601216)	......T... ......T... ...T.TG.C
19	(EF601217)	.....GT... .C.A..T... ...T.T...
20	(EF601218)	......T... ...A..T... ...T.T.C.
Western North Atlantic Ocean		
23	(EF601219)	C....G.TG. .C..... T...AT...
24	(EF601220)	C.C.TG.TG. .C......C. T....T...

Table 3. Sequence statistics for false killer whale mitochondrial DNA control region sequences for the (a) broad-scale (Fig. 3a) and (b) fine-scale (Fig. 3b) stratifications used for the population structure analyses.

(a)

Sequence characteristics	All samples (*n*=151)	Hawai'i Insular (*n*=81)	Central North Pacific Ocean (*n*=13)	Eastern North Pacific Ocean (*n*=39)
Number of groups sampled	63	21	10	14
Number of haplotypes	22	3	5	6
Haplotypic diversity (*h*)	0.781 (+/- 0.028)	0.355 (+/- 0.054)	0.6282 (+/- 0.1431)	0.686 (+/- 0.048)
Nucleotide diversity (π)	0.0036 (+/- 0.002)	0.0008 (+/- 0.0007)	0.0029 (+/- 0.0018)	0.0024 (+/- 0.0015)

(b)

Sequence characteristics	Hawai'i Insular (n=81)	Hawai'i Pelagic (n=9)	Mexico (n=19)	Panama (n=15)	American Samoa (n=6)
Number of groups sampled	21	9	4	2	3
Number of haplotypes	3	3	3	2	2
Haplotypic diversity (*h*)	0.355 (±0.054)	0.556 (±0.165)	0.444 (±0.124)	0.343 (±0.128)	0.333 (±0.215)
Nucleotide diversity (π)	0.0008 (±0.0007)	0.0033 (±0.0022)	0.0021 (±0.0014)	0.0015 (±0.0011)	0.0018 (±0.0014)

Six of seven groups with more than one haplotype included females with two different haplotypes. These were Hawai'i insular groups 3, 8, 10, and 11, and Eastern North Pacific groups 4 and 10 (Table 1). Whether the groups sampled were stable or temporary aggregations is unknown, but the data suggest that this species' social structure is not strictly matrilineal.

For the Hawai'i insular stratum, neither Tajima's D (D=-0.816, p=0.238) nor Fu's Fs (F=1.778, p=0.814) provided evidence of population expansion or bottleneck, as neither was significantly different from zero.

Observed heterozygosity and allelic richness for the eight nDNA markers were similar across all strata (Table 4). The samples from the Hawai'i insular stratum possessed more alleles, on average than the other strata in the fine-scale stratification, as would be expected given its much larger sample size. The number of alleles detected at the different loci ranged from 5 (Ttr34) to 13 (KWM2at; Table 4).

Table 4. Gene diversity for the nuclear DNA data set is presented by stratum (Fig. 3) along with the number of alleles and observed heterozygosity for each locus. Allelic richness was calculated using a minimum sample size of ten in the broad-scale stratification and four in the fine-scale stratification.

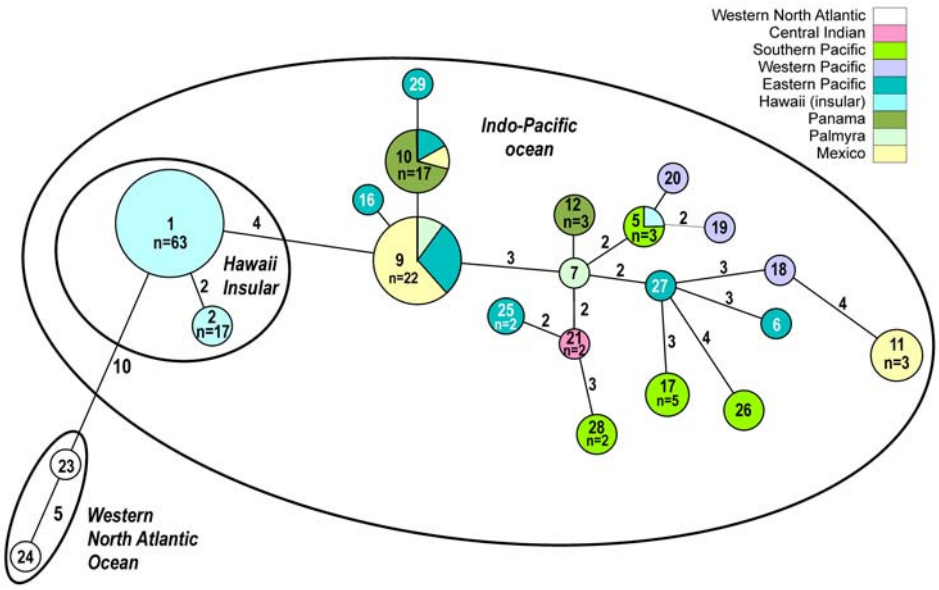
Population	n	Mean number of alleles	H_o	Mean Allelic richness
Broad-scale strata (Fig. 3a)				
Hawai‘i Insular	69	7.13	0.688±0.207	5.44±1.45
Central North Pacific	13	6.13	0.674±0.152	5.83±2.29
Eastern North Pacific	36	8.25	0.726±0.132	5.87±1.64
Fine-scale strata (Fig. 3b)				
Hawai‘i Insular	69	7.13	0.688±0.207	3.940±0.94
Mexico	19	6.88	0.733±0.157	4.115±0.89
Panama	12	5.75	0.693±0.278	3.806±1.17
Hawai‘i Pelagic	9	5.50	0.636±0.175	4.026±1.13
American Samoa	6	5.00	0.729±0.235	4.222±1.35
Locus				
D12t		9	0.546	9.00
EV94t		12	0.770	11.93
KWM12at		12	0.860	12.00
KWM2at		13	0.797	12.85
KWM2b		6	0.307	5.93
Ttr11		11	0.778	10.89
Ttr34		5	0.711	5.00
Ttr48		10	0.805	10.00

Phylogeographic structure

The minimum spanning network shows the relationship among haplotypes, their frequency and geographic distribution. Phylogeographic concordance is evident in the distribution of haplotypes (Fig. 4). That is, each oceanic region has a unique set of haplotypes, and within the Pacific Ocean basin, two haplotypes are found only in Hawai‘i insular false killer whales. Five additional haplotypes (i.e., haplotypes 25-29) were identified among the samples added to the Chivers et al. (2007) data set, and all are closely related to other Indo-Pacific Ocean haplotypes. A minimum of one to four nucleotide differences separates the most closely related haplotypes within the Indo-Pacific Ocean, and a minimum of 10 nucleotide differences separated the most closely related Indo-Pacific Ocean and Atlantic Ocean haplotypes.

There were a few shared haplotypes observed between sampling sites within the Pacific Ocean (Table 5). Specifically, most animals sampled within the Palmyra Atoll EEZ had the most common haplotype identified from samples collected off Mexico (haplotype 9; Table 5). One group sampled ~12 km off the island of Hawai‘i (i.e., group 4 in Fig. 2) included individuals with Hawai‘i insular haplotypes 1 and 2 and haplotype 5, a haplotype also identified from animals sampled off Northern Australia. Three distinctive individuals from this group were matched to known individuals in the Hawai‘i insular photo-identification catalog. Two of these photographically matched individuals had biopsies with the Hawai‘i insular haplotype 1.

(a)



(b)

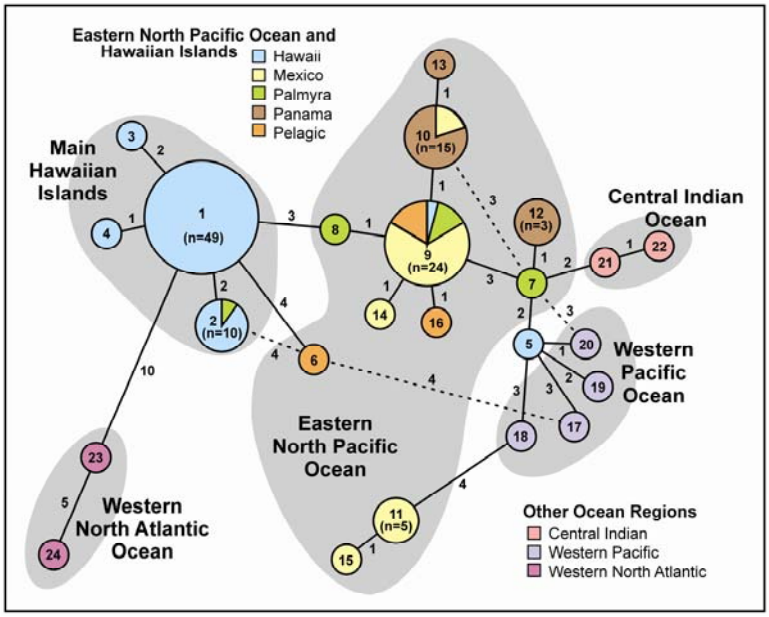


Figure 4. (a) Minimum spanning network for the 22 haplotypes identified for false killer whales in this study. Each haplotype is identified by a number, which corresponds to the numbers in Table 2, and the observed frequency is shown if greater than 1. Each connecting branch is labeled with the minimum number of base pair changes if greater than 1. (b) The minimum spanning network published as Figure 3 in Chivers et al. (2007). Dashed lines indicate alternate links.

Seven samples were collected by fishery observers aboard longline fishing vessels, and all had Pacific Ocean haplotypes. Five of the six sampled within or relatively near the EEZ of Hawai‘i (Fig. 1b) had the most common haplotype seen among animals sampled in the ENP (i.e., haplotype 9) and the sixth animal had haplotype 6. The seventh animal was sampled by a fishery observer around American Samoa and had haplotype 26 (Table 5; Fig. 3).

Table 5. Haplotype frequencies for false killer whales from eastern Pacific Ocean sampling locales after duplicates were removed. Samples included in the Hawaiian Island columns were collected from Hawai‘i insular false killer whales. The Central North Pacific stratum is the Hawai‘i pelagic and Palmyra strata combined plus one additional sample (haplotype 16) sampled from about 25^{o} N, -135^{o} W (Fig. 1b). Six samples collected by fishery observers are included in the Hawai‘i pelagic stratum (haplotypes 6 (n=1) and 9 (n=5)), and one sample collected by fishery observers is included in the American Samoa stratum (haplotype 26 (n=1)).

Haplotype ID number	O‘ahu, HI (*n*=26)	Maui, HI (*n*=12)	Hawai‘i, HI (*n*=43)	Hawai‘i Pelagic (*n*=9)	Palmyra (*n*=3)	Mexico (*n*=19)	Panama (*n*=15)	American Samoa (*n*=6)
1	24	9	30					
2	2	3	12					
5			1					
6				1				
7					1			
9				6	2	14		
10						2	12	
11						3		
12							3	
17								5
25				2				
26								1

Assignment test

The Hawai‘i insular false killer whales could not be excluded as a possible population of origin for any of the samples collected from group 4 (Fig. 2). In contrast, at least one sample from each of the other strata in the fine-scale stratification could be excluded from having originated in the Hawai‘i insular stratum (Table 6). Relatively large assignment probabilities for individuals in distant strata to Hawai‘i insular false killer whales suggests that the power of assignment test using only eight nDNA markers is low for any single individual.

Table 6. Assignment probabilities of individuals to the Hawai‘i insular stratum. The column ‘Stratum’ indicates the location at which each individual was sampled. For individuals with assignment probabilities less than 0.01 (shown in bold), the Hawai‘i insular false killer whales can be excluded as a possible population of origin.

Individual ID	Stratum	Assignment Probability	# homozygous genotypes
49043	HI group 4	0.677	0
49046	HI group 4	0.13	1
49047	HI group 4	0.097	1
49048	HI group 4	0.824	0
49049	HI group 4	0.538	1
49051	HI group 4	0.031	1
49052	HI group 4	0.095	1
41854	HI Pelagic	0.617	0
41855	HI Pelagic	0.125	1
49097	HI Pelagic	0.187	1
49098	HI Pelagic	0.022	0
53477	HI Pelagic	0.019	1
73895	HI Pelagic	0.219	1
73896	HI Pelagic	**0**	**0**
73897	HI Pelagic	0.216	0
79904	HI Pelagic	0.064	0
16136	Panama	**0.008**	**0**
16138	Panama	0.121	0
16139	Panama	0.101	1
16140	Panama	0.091	0
16142	Panama	0.067	0
16143	Panama	0.113	0
16144	Panama	0.037	0
16146	Panama	0.215	0
16148	Panama	0.053	0
18447	Mexico	0.043	0
18448	Mexico	**0.009**	**0**
18454	Mexico	0.012	1
18455	Mexico	0.521	0
18457	Mexico	0.327	0
18458	Mexico	0.017	1
18459	Mexico	0.066	0
18461	Mexico	0.05	0
18462	Mexico	**0.01**	**1**
18463	Mexico	**0**	**0**
18464	Mexico	0.17	0
18465	Mexico	0.05	0
45817	American Samoa	0.289	0
74710	American Samoa	0.03	1
79762	American Samoa	0.101	1
79763	American Samoa	**0.001**	**1**
79764	American Samoa	0.019	0
79765	American Samoa	0.104	0

Genetic differentiation

Analysis of mtDNA data – We found evidence of statistically significant genetic differentiation among strata. Global tests of differentiation revealed significant genetic differentiation between strata overall for the broad- and fine-scale stratifications (Fisher's exact *p*-value <0.0001 for both stratifications), rejecting the global null hypothesis of no population structure. The global test of genetic differentiation was not statistically significant for the inter-island stratification (Fisher's exact *p*-value = 0.165). In the broad-scale stratification, all pairwise comparisons of the Hawai'i insular stratum to other strata were statistically significant (Table 7). Similarly, in the fine-scale stratification, all pairwise comparisons were statistically significant except for the comparison of Mexico to Hawai'i pelagic (Table 8). Genetic divergence (Φ_{ST}) between the Hawai'i insular stratum and other strata examined ranged from 0.687 to 0.856 (Tables 7 and 8).

For the inter-island stratification, only the comparison between O'ahu and Hawai'i was marginally statistically significant (Table 9). These results differ from those presented in Chivers et al. (2007; their Table 5) where all pairwise comparisons were statistically significant when the full data set, including duplicate individuals, was used. We did not detect any evidence of sex-biased dispersal of living immigrants using the bootstrap analysis (Table 10).

Table 7. Mitochondrial DNA results for the broad-scale stratification (Fig. 3a): Φ_{ST}, with p-values in parentheses, below the diagonal, and Fisher exact test p-values above the diagonal. P-values <0.05 are shown in bold.

Putative population	Hawai'i Insular (*n*=81)	Central North Pacific (*n*=13)	Eastern North Pacific (*n*=39)
Hawai'i Insular	--	**<0.0001**	**<0.0001**
Central North Pacific	**0.736 (<0.0001)**	--	**0.0004**
Eastern North Pacific	**0.687 (<0.0001)**	**0.123 (0.0048)**	--

Table 8. Mitochondrial DNA results for analyses of the fine-scale stratification (Fig. 3b). Φ_{ST}, with p-values in parentheses, are below the diagonal, and Fisher exact test p-values are above the diagonal. P-values <0.05 are shown in bold.

Putative population	Hawai‘i Insular (*n*=81)	Hawai‘i Pelagic (*n*=9)	Mexico (*n*=19)	Panama (*n*=15)	American Samoa (*n*=6)
Hawai‘i Insular	--	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Hawai‘i Pelagic	**0.7363 (<0.0001)**	--	0.0736	**<0.0001**	**0.0003**
Mexico	**0.7437 (<0.0001)**	0.0409 (0.2039)	--	**<0.0001**	**<0.0001**
Panama	**0.7920 (<0.0001)**	**0.3624 (<0.0001)**	**0.4026 (<0.0001)**	--	**<0.0001**
American Samoa	**0.8560 (<0.0001)**	**0.4968 (0.0004)**	**0.6111 (<0.0001)**	**0.7492 (<0.0001)**	--

Table 9. Mitochondrial DNA results for the inter-island comparisons of false killer whales sampled from the Hawai‘i insular false killer whales. Φ_{ST}, with p-values in parentheses, are below the diagonal, and Fisher exact test p-values are above the diagonal. P-values <0.05 are shown in bold.

Putative population	O‘ahu (*n*=26)	Maui (*n*=12)	Hawai‘i (*n*=43)
O‘ahu	--	0.3054	0.0589
Maui	0.0661 (0.3013)	--	1.0000
Hawai‘i	**0.0853 (0.0462)**	-0.0498 (0.9998)	--

Table 10. Differences in F_{ST} calculated from mitochondrial DNA between females and males are presented for comparisons a) within the main Hawaiian Islands Hawai'i insular false killer whales only, and b) among Mexico, Panama, and Hawai'i insular false killer whales. P-values from bootstrap analysis comparing the difference between the sexes to the null hypothesis of no sex-biased dispersal are shown in parentheses.

(a)

Putative population	O'ahu	Maui
O'ahu	--	
Maui	0.084 (0.940)	--
Hawai'i	0.125 (0.599)	0.019 (0.767)

(b)

Putative population	Hawai'i Insular	Mexico
Hawai'i Insular	--	
Mexico	0.026 (0.843)	--
Panama	0.031 (0.811)	0.078 (0.844)

No differences in significant versus non-significant results were found in any of the comparisons of the broad- or fine-scale stratifications when seven additional samples were excluded as potential duplicates (Appendix 1). In the inter-island stratification, the additional exclusions resulted in the comparison between O'ahu and Hawai'i becoming non-significant.

Analysis of nuclear marker data – Global tests of differentiation revealed significant differentiation within the nDNA data set, leading to rejection of the global null hypothesis of no population structure, for both the broad-scale (χ^2 $p < 0.0001$) and fine-scale (χ^2 $p < 0.0001$) stratifications. The global test was not statistically significant for the inter-island stratification (χ^2 $p = 0.189$). We found statistically significant evidence of genetic differentiation among putative populations for most pairwise comparisons in the broad- and fine-scale stratifications, with Hawai'i insular false killer whales significantly differentiated from all other strata using the χ^2 test (Tables 11a and 12a). In the broad-scale stratification, CNP and ENP were also significantly differentiated from each other (Table 11). In the fine-scale stratification all pairs of strata except American Samoa/Hawai'i pelagic were significantly differentiated, according to the χ^2 test (Table 12a). The estimates of divergence between Hawai'i insular false killer whales and other strata ranged from 0.0199 to 0.048 for F_{ST} and from 0.009 to 0.039 for Jost's D (Tables 11b and 12b). Fewer comparisons were found to be statistically significant for the permutation tests of F_{ST} and Jost's D, which is consistent with the fact that such tests are less powerful than the χ^2 test (Goudet et al. 1996). The inter-island comparisons revealed a statistically significant difference for only the comparison of Hawai'i to O'ahu (Table 13). No evidence of sex biased dispersal was detected (Table 14).

Table 11. Pairwise comparisons of the nuclear DNA data set for the broad-scale stratification (Fig. 3a). (a) X^2 p-values. (b) F_{ST} values are below the diagonal, Jost's D above the diagonal. P-values are given in parentheses and those <0.05 are shown in bold.

(a)

Putative population	Hawai'i Insular (*n*=69)	Central North Pacific (*n*=13)	Eastern North Pacific (*n*=36)
Hawai'i Insular	--		
Central North Pacific	**0.0001**	--	
Eastern North Pacific	**0.0001**	**0.0090**	--

(b)

Putative population	Hawai'i Insular (*n*=69)	Central North Pacific (*n*=13)	Eastern North Pacific (*n*=36)
Hawai'i Insular	--	**0.0188 (0.0185)**	**0.0395 (0.0001)**
Central North Pacific	**0.0215 (0.0070)**	--	**0.0111 (0.0379)**
Eastern North Pacific	**0.0261 (0.0001)**	**0.0174 (0.0146)**	--

Table 12. Pairwise comparisons of the nuclear DNA data set for fine-scale stratification (Fig. 3b). (a) X^2 p-values. (b) F_{ST} values are below the diagonal, Jost's D above the diagonal. P-values are given in parentheses and those <0.05 are shown in bold.

(a)

Putative population	Hawai'i Insular (*n*=69)	Hawai'i Pelagic (*n*=9)	Mexico (*n*=19)	Panama (*n*=12)	American Samoa (*n*=6)
Hawai'i Insular	--				
Hawai'i Pelagic	**0.0002**	--			
Mexico	**0.0001**	**0.0091**	--		
Panama	**0.0001**	**0.0245**	**0.0320**	--	
American Samoa	**0.0003**	0.1501	**0.0002**	**0.0006**	--

(b)

Putative population	Hawai'i Insular (*n*=69)	Hawai'i Pelagic (*n*=9)	Mexico (*n*=19)	Panama (*n*=12)	American Samoa (*n*=6)
Hawai'i Insular	--	0.0090 (0.0809)	**0.0232 (0.0039)**	**0.0168 (0.0225)**	0.0124 (0.0682)
Hawai'i Pelagic	**0.0199 (0.0318)**	--	0.0052 (0.1693)	**0.0631 (0.0012)**	0.0051 (0.2216)
Mexico	**0.0226 (0.0006)**	0.0179 (0.0842)	--	0.0100 (0.0531)	**0.0312 (0.0292)**
Panama	**0.0484 (0.0001)**	**0.0402 (0.0053**	**0.0174 (0.0399)**	--	**0.1963 (0.0001)**
American Samoa	0.0222 (0.0658)	0.0089 (0.3290)	**0.0373 (0.0203)**	**0.0809 (0.0005)**	--

Table 13. Pairwise inter-island comparisons of the nuclear DNA data set for false killer whales sampled from the Hawai'i insular false killer whales. (a) X^2 p-values. (b) F_{ST} values are below the diagonal, Jost's D above the diagonal. P-values are given in parentheses and those <0.05 are shown in bold.

(a)

Putative population	O'ahu (n=19)	Maui (n=12)	Hawai'i (n=38)
O'ahu	--		
Maui	0.1993	--	
Hawai'i	**0.0259**	0.7612	--

(b)

Putative population	O'ahu (*n*=19)	Maui (*n*=12)	Hawai'i (*n*=38)
O'ahu	--	0.0021 (0.2045)	**0.0191 (0.0140)**
Maui	0.0129 (0.1293)	--	-0.00003 (0.4889)
Hawai'i	**0.0273 (0.0007)**	-0.0035 (0.6309)	--

Table 14. Difference in F_{ST} calculated from nuclear DNA between females and males a) within each of the main Hawaiian Islands for Hawai'i insular false killer whales only, and b) within Mexico, Panama, and Hawai'i insular strata. P-values from bootstrap analysis comparing the difference between the sexes to the null hypothesis of no sex-biased dispersal are shown in parentheses.

(a)

Putative population	O'ahu	Maui
O'ahu	--	
Maui	0.027 (0.521)	--
Hawai'i	0.006 (0.845)	0.026 (0.374)

(b)

Putative population	Hawai'i Insular	Mexico
Hawai'i Insular	--	
Mexico	0.006 (0.813)	--
Panama	0.045 (0.106)	0.032 (0.207)

No differences in significant versus non-significant results were found in any comparisons of the broad-scale comparisons when seven samples were excluded as potential duplicates (Appendix 1). In the fine-scale comparisons, there were no differences in significant versus non-significant results for the χ^2 tests. However, the *p*-values changed from just less than 0.05 to just over 0.05 for Hawai'i insular/Hawai'i pelagic and Mexico/Panama for F_{ST} and from just over 0.05 to just less than 0.05 for Mexico/Panama for D. Note that the changes in significance for the Mexico/Panama comparison are strictly due to stochasticity in the permutation, as these strata are identical between the two analysis sets. In the inter-island strata, the *p*-values for both χ^2 and D between O'ahu and Hawai'i went from significant in the main analysis to non-significant in the sensitivity analysis, while the same comparison remained significant for F_{ST} in both analyses (Appendix 1)

Effective population size –The effective population size for Hawai'i insular false killer whales was estimated as 44.3 (95% CI = 31.2-67.2). Sample sizes relative to likely abundances for the other strata were insufficient to make reliable estimates of effective population size.

Bottleneck **–** BOTTLENECK detected evidence of a recent decline in N_e in Hawai'i insular false killer whales ($P = 0.015$), with all eight loci exhibiting heterozygosity excess. Seven out of eight loci also exhibited heterozygosity excess in the Mexico stratum, but the overall result was not statistically significant ($P = 0.103$).

Discussion

The addition of new samples and nDNA data confirmed the demographic independence of Hawai'i insular false killer whales with significant differentiation from both broad- and fine-scale strata using both mtDNA and nDNA. We focus our discussion on the implications of our results for understanding the evolutionary importance of this distinct group of false killer whales. However, we first caution the reader that considerable uncertainties remain for several reasons, including large gaps in the sample distribution, small sample sizes in all strata except the Hawai'i insular stratum, and many samples collected from only a few groups (Table 1). The ideal sample distribution to evaluate Hawai'i insular false killer whales in an evolutionary context would include good sampling of nearby pelagic waters (preferably of nearly equal sample size) and sampling from a similar archipelago (like the Cook Islands). Given that false killer whales are a naturally uncommon species, many decades will likely be needed to collect samples that adequately represent their distribution. For example, in 4 months of surveying the EEZ of Hawai'i for cetaceans, only two sightings were made and no biopsies were obtained due to rough water conditions (Jay Barlow, pers. comm.[1])Also of concern, but to a much lesser degree, is the limited number of genetic markers used. Given the low genetic diversity in false killer whales, data for additional microsatellite loci and the full mitogenome could enhance our ability to draw conclusions about the evolutionary relationships among populations.

We begin with interpretation of the mtDNA data. There are two hypotheses consistent with the observed data that all but one Hawai'i insular false killer whale have one of only two closely related haplotypes: 1) Hawai'i was colonized by a group containing a single haplotype

[1] Jay Barlow, Protected Resources Division, Southwest Fisheries Science Center, National Marine Fisheries Service, 3333 N. Torrey Pines Court, La Jolla, CA 92037.

and the second haplotype evolved in Hawai‘i, or 2) Hawai‘i was colonized by a group containing multiple haplotypes and the two observed haplotypes drifted to high frequency. Both hypotheses are consistent with a group that is closed or nearly closed to immigration. Not finding these haplotypes elsewhere, recognizing the sampling limitations, is consistent with both hypotheses. The only other strata with relatively good sample sizes (larger than 15) are Mexico and Panama. These strata each have common haplotypes that differ by many base pairs from one another. That is, haplotype 9 is most common in Mexico followed by haplotype 11, which differs by 12 base pairs, while haplotype 10 is most common in Panama (Table 5; Fig. 4). Although limited, the evidence suggests local evolution within Hawai‘i is more plausible than colonization by closely related haplotypes.

The Atlantic Ocean is not well represented in this study, but the genetic differentiation observed is consistent with what would be expected when comparing tropical species between these ocean basins (Davies 1963; Perrin et al. 1981; Chivers et al. 2005). These results are also consistent with the morphological differences documented between false killer whales sampled off Australia and Scotland (Kitchener et al. 1990), and between Japan and South Africa (Ferreira 2008). Resolving the magnitude of population structure for false killer whales worldwide and whether subspecies exist will require analyses of data that much better represent the whales' distribution.

The magnitude of mtDNA differentiation is large (i.e., Φ_{ST} >0.68) for both the broad- and fine-scale analyses conducted here. The one-migrant-per-generation rule of thumb ($Nm = 1$, where N is the effective population size and m is the migration rate per generation) developed by geneticists is approximately the level of gene flow needed to maintain genetic diversity within a population (Mills and Allendorf 1996). This rule has been used in terrestrial applications to determine when fragmented populations require genetic rescue from nearby populations. The rule is also used in conservation to highlight populations that experience low gene flow and hence have conditions that would be conducive to local adaptation given even weak selection. The argument is that high gene flow from neighboring populations not experiencing the local conditions would swamp weak selection. In our case, we use the one-migrant-per-generation as a rule of thumb to indicate the conditions conducive for selection to outweigh gene flow from neighboring populations. This rule is used by conservation geneticists in two ways: 1) to decide how much gene flow is needed to overcome the potential negative effects of habitat fragmentation, and 2) to decide whether a group of individuals is so genetically isolated that it is possible that local adaptation could have occurred. In our case, we are interested in the second application. We calculated measures (F_{ST}, Φ_{ST} and Jost's D) that are commonly used to indicate the level of differentiation using units of migrants/generation. For mtDNA, one-migrant-per-generation corresponds to F_{ST} or Φ_{ST} of 0.33. All comparisons of Hawai‘i insular false killer whales to other strata have much higher values indicating much lower estimated gene flow (Tables 7 and 8). For nDNA, the rule corresponds to F_{ST} or Jost's D of 0.2, assuming that the mutation rate is much less than the migration rate. Despite finding strong significant differences between Hawai‘i insular false killer whales and all other strata, the nDNA results (Tables 11 and 12) are all well below this value.

Understanding the apparently different magnitude of signals between mtDNA and nDNA with respect to the potential evolutionary significance of the Hawai‘i insular false killer whales will remain speculative until sample gaps are filled and more is understood about the social structure of false killer whales and the behavior of the differentiation statistics for microsatellites,

which violate both the assumptions of low mutation rate and infinite alleles. Below are a series of plausible hypotheses that cannot be discounted given current data:

- There is a low level of male-mediated gene flow that was not apparent because there is insufficient sampling of nearby groups of false killer whales and/or the test for male-mediated gene flow can only detect first-generation male migrants
- The magnitude of nDNA differentiation is under-estimated because of the high mutation rate of microsatellites.
- The magnitude of differentiation is not inconsistent with cases where selection has been shown to be strong enough for local adaptation.

The potential of male-mediated gene flow is a likely explanation for the apparent differences in the magnitude of differentiation between mtDNA and nDNA. The tests done in this paper would only detect immigrant males but not gene flow from males that are not present in the current sample (i.e. from past generations or by visitors). Thus, the negative finding does little to rule out the possibility of male-mediated gene flow. However, there are several non-trivial difficulties with interpreting the statistics of differentiation using a marker that violates the assumption of infinite alleles and has a high mutation rate (like microsatellites). It is known that microsatellites can be misleading and over-estimate the amount of gene flow (Balloux et al. 2000). When mutation rate is accounted for in the equations for F_{ST}, the expected value for one-migrant-per-generation is between 0.031 and 0.075 for mutation rates between 0.01 and 0.001, which should bracket the mutation rates for microsatellites. Thus, our observed values are close to this range, and there may be rather little difference between differentiation results for mtDNA and nDNA. However, a more fundamental issue to consider is whether statistics like F_{ST} actually measure differentiation (Jost 2008). Jost makes a compelling argument that as within population variance gets large (which is correlated with using a marker with a high mutation rate), the ratio of within population variance to total variance becomes necessarily small regardless of the amount of differentiation between populations.

Jost (2008) designed the statistic D so that it is independent of within-population variance and therefore should represent a true measure of differentiation. Nonetheless, significant difficulties remain with properly interpreting values of D. Like F_{ST}, D also depends on mutation rate. Furthermore, it assumes an infinite allele model of mutation, which does not apply to microsatellites. Further work needs to be done with simulations to determine the impact of violating this assumption and to provide insight into how D should be interpreted for microsatellite data.

The one-migrant-per-generation rule may also be conservative when using neutral markers. Population genetic theory indicates that the possibility of local adaptation depends on the relationship between *m* (the migration rate) and *s* (the selection coefficient), not the relationship between *Nm* and *s*. In general, local adaptation occurs when $m << s$. If *N* is large, then *Nm* can be large (>>1) and still have *m* be small enough to make local adaptation plausible (Hedrick 2000). In the case of Hawai‘i insular false killer whales we have no data to estimate *s*. There are also numerous empirical studies with relatively low F_{ST} values estimated from neutral genetic markers where population differentiation is known to be strong and selection is or is likely to be strong. Examples include chum salmon populations across the Pacific Rim (Beacham et al. 2009), Chinook salmon (O'Malley et al. 2007), different chromosome races of common shrews (Balloux et al. 2000) and several studies of *Drosophila* (Berry and Kreitman

1993, Ford and Aquadro 1996). These examples serve as a reminder that local adaption is accomplished via coding markers and that neutral markers will always underestimate true differentiation if selection is present.

The presence of two distinct, closely related haplotypes in Hawai‘i insular false killer whales is consistent with this habitat not being colonized regularly from other areas. This pattern differs from stocks of Hawaiian common bottlenose, spinner (*Stenella longirostris*) and pantropical spotted (*S. attenuata*) dolphins that all have minimum spanning networks suggesting multiple colonization events or ongoing gene flow from a larger, pelagic population (Andrews et al. 2006, 2010; Courbis et al. 2010; Martien et al. 2006). The pattern of nearly all individuals with one of only two closely related haplotypes shown in Hawai‘i insular false killer whales is consistent with a strong social system that excludes immigrants or strong habitat specialization that makes survival of immigrants unlikely (or both). One single individual was found among Hawai‘i insular false killer whales with haplotype 5. Although the individual with haplotype 5 does not have a photograph to connect it directly to the Hawai‘i insular social network of false killer whales, it was sampled within a group with such links and cannot be excluded using assignment tests as being part of the Hawai‘i insular group. The individual was a male. Given the low power of the current assignment test (i.e., only eight nDNA microsatellite markers) the possibility of immigration (permanent membership as a Hawai‘i insular false killer whale but with an origin outside the group) cannot be ruled out. Likewise, the possibility that this individual was a visitor from the pelagic population cannot be excluded nor can we rule out that this individual is a full Hawai‘i insular false killer whale with a rare haplotype. The rare haplotype is sufficiently distantly related such that it seems most plausible it originated from a separate colonization event (i.e., immigrants are accepted on rare occasions).

There are several other examples of cetaceans that have morphologically and genetically differentiated units occupying adjacent coastal or island habitats and pelagic habitats. These include coastal or island populations of pantropical spotted dolphin (Douglas et al. 1984; Escorza et al. 2005), common and Indo-Pacific bottlenose dolphins (Walker 1981; Mead and Potter 1995; Hoelzel et al. 1998b; Wang et al. 1999; Lowther 2006; Rosel et al. 2009), spinner dolphin (Norris et al. 1994; Galver 2002; Andrews et al. 2006, 2010), and short-finned pilot whale (SWFSC unpublished data[2]). Knowledge about false killer whales occupying other island and coastal habitats around the world would be valuable to interpreting the results presented here.

We estimate that the effective population size of Hawai‘i insular false killer whales is less than 50 animals. This population is probably naturally small with a strong social structure that limits genetic diversity. Nonetheless, such a low estimate of N_e is cause for concern, as domestic animals have been shown to start displaying bad genetic effects (lethal or semi-lethal traits) when effective population size reaches less than 50 individuals (Franklin 1980). However, there are several potential sources of bias in our estimate. The method we used is known to have a slight (<5%) negative bias (Waples 2006). Furthermore, it was developed and tested under the assumption of a closed population with non-overlapping generations. If the population is not completely closed, but rather receives immigrants from other populations, the estimate will be positively biased due to a Wahlund effect created by the presence of first-generation immigrants

[2] Chivers, S. J., R. G. LeDuc, and R. W. Baird. 2003. Hawaiian island populations of false killer whales and short-finned pilot whales revealed by genetic analysis. Abstract for the 15th Biennial Conference on the Biology of Marine Mammals, Greensboro, NC, USA. December 2003.

in the population. The amount of linkage disequilibrium introduced by this effect is small (Waples and Smouse 1990) and therefore unlikely to significantly impact estimates of N_e. Nonetheless, the magnitude of any potential bias due to immigration has not yet been quantified (Waples 2006). Similarly, the bias, if any, introduced by overlapping generations has also not been well studied, although Waples (2006) notes that analyses of populations with overlapping generations will estimate the effective number of breeders that produced the sample, which is related to N_e.

The estimate of N_e produced by LDNe is influenced by the effective size of the population in the generations immediately prior to the collection of samples. Although no data are available for calculating trends in abundance for Hawai‘i insular false killer whales, observational data suggest abundance may have declined precipitously over the last two decades (Baird 2009; Reeves et al. 2009). We also detected strong evidence in the nDNA data set of a recent reduction in the abundance of Hawai‘i insular false killer whales. BOTTLENECK detected an excess of heterozygosity at all eight nDNA loci we examined, which is indicative of a population that is out of equilibrium due to a recent decline in effective abundance. Though estimates of N_e based on linkage disequilibrium stabilize within a few generations following changes in population size (Waples 2006), the fact that the decline in effective population size of this population likely occurred less than a generation ago means that the estimate of N_e we present likely represents the pre-decline value. Thus, our estimate of N_e likely over-estimates the effective size of the current population.

We did not detect any evidence of a decline in effective abundance for the samples in our Mexico stratum, suggesting that this population has maintained a constant size over the recent evolutionary past. Though there are no observational or demographic data to suggest a decline for any false killer whales other than Hawai‘i insular false killer whales, few data are available. The limited number of samples (n=19) available from Mexico, as well as the small number of nuclear markers included in our study, likely result in very low power to detect declines anywhere outside the Hawai‘i insular stratum (Cornuet and Luikart 1996).

Our study demonstrates the genetic distinctness of Hawai‘i insular false killer whales. We have acknowledged the sampling limitation of our study, and here we add that these could affect the genetic characterization of Hawai‘i insular false killer whales if there are as yet unsampled members of the population. We mention this specifically because there was a group of false killer whales photographed off Kaua’i that were not connected with the photographic social network of known Hawai‘i insular false killer whales and had no biopsies taken (Baird 2009). If an additional population, or populations, of false killer whales live around Kaua‘i or the Northwest Hawaiian Islands, their information is not included in the N_e or other genetic-based estimates. However even acknowledging the sampling limitations of our study, the limited genetic diversity and small size of this population that may have recently declined (Baird 2009; Reeves et al. 2009) are causes for concern.

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Appendix 1
Sensitivity analyses

Comparisons of nuclear DNA (nDNA) genotypes revealed seven pairs of false killer whale samples that were possible duplicates (Table A1). Each pair had alleles that differed for at least two markers and in most cases the differences fit the pattern of allelic dropout. Two of these pairs also had different mitochondrial DNA (mtDNA) haplotypes. Mis-matched mtDNA and nDNA data were reviewed from raw data, and in several cases, the data were re-generated in the laboratory. In all of these cases, the markers remained different. The main data set was modified as follows to create the mtDNA and nDNA data sets for these analyses. The mtDNA data set was created by excluding one sample from each pair of samples except that both samples were excluded for the two pairs of samples with different haplotypes. The nDNA data set was created by setting the allele calls that differed to null and excluding one of each pair. However in one case, the allele calls set to null resulted in both members of the pair having more than three missing genotypes and thus both samples were excluded. The exception was the pair 23320/33903. In this case, 33903 was excluded from the nDNA sensitivity data set, while 23320 was retained, but with its genotype at KWM2at (i.e., the mis-matching genotype) set to null.

The remaining tables in this appendix present the results for analyses of the mtDNA (Tables A2-A4) and nDNA (Tables A5-A7) data sets using the broad, fine, and inter-island stratifications described in the Methods section of the report. The results of these analyses were compared to those described in the the report to illustrate the influence of including these potential duplicate samples.

Table A1. Haplotype (i.e., HapID), sex and genotype data are presented for pairs of samples with genotype data indicating they may represent the same individual. Each sample is referred to by its SWFSC Protected Species Tissue Archive Accession Number (i.e., LabID). Data discrepancies are highlighted in bold text with genotype differences that might be allelic dropout shown in italics. The two "use" columns at the far right indicate whether a sample was excluded from a data sets (i.e., Use = No).

LabID	HapID	Sex	D12t	EV94t	KWM12at	KWM2at	KWM2b	Ttr11	Ttr34	Ttr48	Use mtDNA	Use nDNA
23320	**2**	F	192194	280284	174192	***146146***	181181	198198	177179	140140	No	Yes
33903	**1**	F	000000	280284	174192	***146168***	181181	198198	000000	140140	No	No
18946	1	**M**	188188	260280	**174184**	146148	181181	***198208***	***179179***	140146	No	No
33887	1	**F**	188188	000000	**176176**	146148	181181	***198198***	***177179***	140146	Yes	No
33903	1	**F**	000000	***280284***	**174192**	146168	181181	198198	000000	140140	No	No
91278	1	**M**	188188	***284284***	**182182**	146168	181181	198198	177177	000000	Yes	No
27453	1	F	188192	***284284***	***184184***	***144148***	177181	**198212**	***179183***	**142146**	Yes	No
27454	1	F	188192	***282284***	***174184***	***144144***	177181	**212218**	***179179***	**138142**	No	No
67156	**28**	M	188188	***282282***	**174186**	146148	181181	204210	179181	***138138***	No	No
72691	**10**	M	188188	***260282***	**182184**	146148	181181	000000	179181	***138140***	No	No
41286	1	M	188192	***260282***	000000	**146148**	181181	**208218**	177181	138138	No	No
30073	1	M	188192	***260260***	180186	**148150**	181181	**200208**	177181	138138	Yes	No

Table A2. For comparison to Table 7: Results of pairwise comparisons of the mitochondrial DNA data set for the broad-scale stratification (Fig. 3a). Φ_{ST}, with p-values in parentheses, are below the diagonal, and Fisher exact test p-values are above the diagonal. P-values <0.05 are shown in bold.

Putative population	Hawai‘i Insular (*n*=75)	Central North Pacific (*n*=13)	Eastern North Pacific (*n*=38)
Hawai‘i Insular	--	**<0.0001**	**<0.0001**
Central North Pacific	**0.726 (<0.0001)**	--	**<0.0001**
Eastern North Pacific	**0.678 (<0.0001)**	**0.115 (0.0069)**	--

Table A3. For comparison to Table 8: Results of pairwise comparisons of the mitochondrial DNA data set for the fine-scale stratification (Fig. 3b). Φ_{ST}, with p-values in parentheses, are below the diagonal, and Fisher exact test p-values are above the diagonal. P-values <0.05 are shown in bold.

Putative population	Hawai‘i Insular (*n*=75)	Hawai‘i Pelagic (*n*=9)	Mexico (*n*=19)	Panama (*n*=15)	American Samoa (*n*=6)
Hawai‘i Insular	--	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Hawai‘i Pelagic	**0.7293 (<0.0001)**	--	0.0548	**<0.0001**	**0.0014**
Mexico	**0.7376 (<0.0001)**	0.0409 (0.2067)	--	**<0.0001**	**<0.0001**
Panama	**0.7876 (<0.0001)**	**0.3624 (<0.0001)**	**0.4026 (<0.0001)**	--	**<0.0001**
American Samoa	**0.8527 (<0.0001)**	**0.4968 (0.0004)**	**0.6111 (<0.0001)**	**0.7492 (<0.0001)**	--

Table A4. For comparison to Table 9: Results of pairwise comparisons of the mitochondrial DNA data set for the inter-island stratification of Hawai'i insular false killer whales. Φ_{ST}, with P-values in parentheses, are below the diagonal, and Fisher exact test P-values are above the diagonal. P-values <0.05 are shown in bold.

Putative population	O'ahu (*n*=22)	Maui (*n*=11)	Hawai'i (*n*=42)
O'ahu	--	0.587	0.139
Maui	-0.032 (0.587)	--	0.766
Hawai'i	0.069 (0.096)	-0.028 (0.600)	--

Table A5. For comparison to Table 11: Results of pairwise comparisons of the nuclear DNA data set for the broad-scale stratification (Fig. 3a). (a) X^2 p-values. (b) F_{ST} values are below the diagonal, Jost's D above the diagonal. P-values are given in parentheses. P-values <0.05 are shown in bold.

(a)

Putative population	Hawai'i Insular (*n*=61)	Central North Pacific (*n*=13)	Eastern North Pacific (*n*=35)
Hawai'i Insular	--		
Central North Pacific	**0.0010**	--	
Eastern North Pacific	**0.0010**	**0.0110**	--

(b)

Putative population	Hawai'i Insular (*n*=61)	Central North Pacific (*n*=13)	Eastern North Pacific (*n*=35)
Hawai'i Insular	--	**0.0149 (0.0300)**	**0.0376 (0.0010)**
Central North Pacific	**0.0182 (0.0170)**	--	0.0085 (0.0619)
Eastern North Pacific	**0.0243 (0.0010)**	**0.0155 (0.0250)**	--

Table A6. For comparison to Table 12: Results of pairwise comparisons of the nuclear DNA data set for fine-scale stratification (Fig. 3b). (a) X^2 p-values. (b) F_{ST} values are below the diagonal, Jost's D above the diagonal. P-values are given in parentheses. P-values <0.05 are shown in bold.

(a)

Putative population	Hawai'i Insular (*n*=61)	Hawai'i Pelagic (*n*=9)	Mexico (*n*=19)	Panama (*n*=12)	American Samoa (*n*=6)
Hawai'i Insular	--				
Hawai'i Pelagic	**0.0020**	--			
Mexico	**0.0010**	**0.0110**	--		
Panama	**0.0010**	**0.0230**	**0.0330**		
American Samoa	**0.0010**	0.1508	**0.0010**	**0.0030**	

(b)

Putative population	Hawai'i Insular (*n*=61)	Hawai'i Pelagic (*n*=9)	Mexico (*n*=19)	Panama (*n*=12)	American Samoa (*n*=6)
Hawai'i Insular	--	0.0075 (0.0879)	**0.0220 (0.0020)**	**0.0926 (0.0010)**	0.0125 (0.0678)
Hawai'i Pelagic	0.0170 (0.0519)	--	0.0052 (0.1548)	**0.0631 (0.0020)**	0.0051 (0.2268)
Mexico	**0.0209 (0.0010)**	0.0178 (0.0949)	--	**0.0100 (0.0410)**	**0.0312 (0.0400)**
Panama	**0.0464 (0.0010)**	**0.0402 (0.0070)**	0.0174 (0.0559)	--	**0.1963 (0.0010)**
American Samoa	0.0215 (0.0660)	0.0089 (0.3267)	**0.0373 (0.0230)**	**0.0809 (0.0020)**	--

Table A7. For comparison to Table 13: Results of pairwise comparisons of the nuclear DNA data set for the inter-island stratification of Hawai‘i insular false killer whales. (a) X^2 p-values. (b) F_{ST} values are below the diagonal, Jost's D above the diagonal. P-values are given in parentheses. P-values <0.05 are shown in bold.

(a)

Putative population	O‘ahu (*n*=14)	Maui (*n*=11)	Hawai‘i (*n*=36)
O‘ahu	--		
Maui	0.1738	--	
Hawai‘i	0.1339	0.9061	--

(b)

Putative population	O‘ahu (*n*=14)	Maui (*n*=11)	Hawai‘i (*n*=36)
O‘ahu	--	0.0035 (0.1838)	0.0091 (0.0619)
Maui	0.0192 (0.0939)	--	-0.0010 (0.6324)
Hawai‘i	**0.0201 (0.0190)**	-0.0074 (0.8072)	--

Appendix 2
Summary of quality control and assurance steps taken to check the nuclear marker data generated for this study

Data generation

Replication – Ten percent of samples were selected randomly from each geographic region as controls for estimating genotyping error rates. In addition to this random replication, controls were run on each plate and all samples collected from within the Palmyra Atoll EEZ and by the longline fishery observers were replicated because they were of interest for the assignment tests. The replicated samples were reviewed for inconsistencies in genotype determination.

Normalization – Microsatellite data were generated over the course of four years and on two different genetic analyzers. Several samples run on the first machine were selected to run on the second machine to allow the allele calls made on the two machines to be normalized (i.e., common allele sizes) using the program Allelogram (Morin et al. 2009b).

Double-blind scoring – Because microsatellite data were generated at two discrete times, allele sizes were called by several different genotypers. To account for variation across genotypers in determining whether an allele size should be recorded and what it should be, all allele sizes were reviewed by an independent genotyper (i.e., one who had never before seen any of the data) after data generation was complete. The independent genotyper re-analyzed all of the genetic analyzer raw data files to determine allele sizes, using the same methodology as the original genotypers. This resulted in a second complete data set that, while based on the same raw data files as the original data set, represented completely independent calls of the raw data. The independent genotyper's allele size calls were then compared to the original calls. This comparison revealed 56 complete genotypes that had been called in one data set but set to null in the other, as well as eight alleles that had different sizes in the two data sets. Of the differing allele sizes, three were samples that were called as homozygotes in one data set and heterozygotes in the other and one was due to a normalization error. The remaining four allele size differences were from two genotypes for which both allele sizes differed between the two data sets. All discrepancies were jointly reviewed and resolved by the independent genotyper and two of the three genotypers who made the original calls. If a genotype could not be resolved upon review, then either the genotype in question was set to null or additional replication in the lab was completed to resolve the difference.

Data archiving – All genetic analyzer runs and allele size calls are archived in the Southwest Fisheries Science Center's genotypes database. As data were reviewed, database records were marked for use if they were deemed to be of good quality.

Data set review

Prior to analyses, the final, complete data set was reviewed to assess marker quality, including the potential of null alleles or allelic drop out, and to identify duplicate samples of the same individual. Procedures outlined by Morin et al. (2009c) were followed.

The program Microchecker version 2.2.3 (Van Oosterhout et al. 2004) was used to examine each marker for allelic dropout and null alleles.

Duplicate samples – The programs DropOut (McKelvey and Schwartz 2005) and MSTools (Park 2001) were used to review the data set for potential duplicate samples. The genotypes of potential duplicates were reviewed along with their mitochondrial DNA haplotype and sex, and if possible, photos. There were 23 samples representing 21 individual animals that matched at all loci, mtDNA sequence and sex. One additional animal was removed from the mtDNA because photographs identified it as a duplicate sample; no genotype data were available for this animal.

There were an additional seven pairs of samples that were considered to be potential duplicate samples. Missing data precluded being able to resolve them as duplicates with certainty. These samples were included in analyses presented in the main text of the paper, and the analyses were repeated omitting them to show their influence on the results. The latter analyses are presented in Appendix 1.

Samples removed – Samples missing data for >25% of the markers or having >70% homozygous genotypes across markers were deemed to be of poor quality and removed from the data set. There were five samples missing data for >25% of the loci, and three samples with >70% homozygous markers.

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